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## Short communication

# Differential localization of G proteins in the opossum vomeronasal system

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 Abstract

$G_{12\alpha}$  and  $G_{o\alpha}$  proteins were immunohistochemically localized, respectively, to the rostral and caudal accessory olfactory bulb of Brazilian opossums. In the vomeronasal organ,  $G_{12\alpha}$ - and  $G_{o\alpha}$ -immunoreactive neurons were located in the middle and basal layers, respectively, of the sensory epithelium. Both G protein antibodies stained the microvillar surface of the epithelium and the nerve bundles in the subepithelial mucosa.

**Keywords:** G protein; Accessory olfactory bulb; Opossum; *Monodelphis domestica*; Differential localization; Vomeronasal organ

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The accessory olfactory bulb (AOB) of the Brazilian gray short-tailed opossum is a heterogeneous structure, distinguishable into anterior and posterior portions on the basis of its chemoarchitecture. The lectin, *Vicia villosa* agglutinin (VVA) [14], the NADPH-diaphorase reaction [17], and antibodies to olfactory marker protein (OMP) [18] stain the anterior AOB significantly more intensely than the posterior AOB.

The AOB of rats and rabbits is also heterogeneous. VVA binding is strong in the posterior 2/3 of the rat AOB with only weak binding in the anterior 1/3 [19].  $G_{12\alpha}$  immunoreactivity is preferentially located in the anterior AOB of rats, while  $G_{o\alpha}$  is preferentially located in the posterior AOB [15]. Monoclonal antibodies (Mab) CC1 and CC6, raised to different sugar moieties, respectively stain the anterior and posterior AOB of the rat [10,11]. In the rabbit two monoclonal antibodies, Mab R4B12 and Mab R5A10, with uncharacterized epitopes, label VN nerve axons terminating in the rostralateral and caudomedial AOB, respectively [8].

The glomerular layer of the AOB of vertebrates is the site of terminations of vomeronasal receptor neurons and is analogous in structure and function to the

glomerular layer of the main olfactory bulb (MOB), the termination site of olfactory bipolar neurons. The glomerular regions of the olfactory bulbs are the first sites in the olfactory and vomeronasal pathways for synaptic interactions, and are sites of considerable convergence of peripheral axons onto postsynaptic cells. It is generally believed that stimulus coding in the main olfactory system is a reflection of the topographical relationship between the receptor cells of the olfactory epithelium and the glomeruli of the MOB [6]. No similar mechanism of coding has been proposed for the accessory olfactory system.

The anatomical differentiation of anterior and posterior regions of the AOB may correspond to functional differences between these two regions. We investigated whether there is a correspondence between the topography of vomeronasal receptor neurons and the topography of the AOB. The experiment described here is an examination of the distribution of  $G_o$  and  $G_i$  immunoreactivity in the vomeronasal epithelium and the AOB of adult gray short-tailed opossums.

G proteins are an integral part of many signal transduction pathways [4] composed of three basic molecular components: a transmembrane receptor protein, an intracellular membrane-associated G protein and an intracellular effector. In most cases, the  $\alpha$  subunit of the heterotrimeric G protein plays a major role in determining the specificity of the interaction of the

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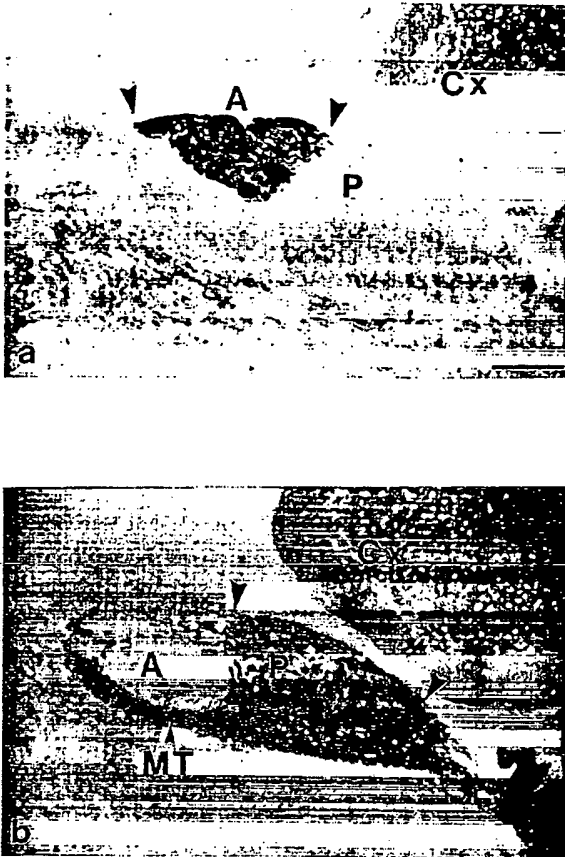


Fig. 1. Parasagittal section through the opossum AOB immunostained with anti-G<sub>12α</sub> (a) and anti-G<sub>oα</sub> (b). Anterior is to the left. The nerve and glomerular layers (large arrowheads) of the anterior AOB (A) are stained with anti-G<sub>12α</sub> (a) and those of the posterior AOB (P) (large arrowheads) are stained with anti-G<sub>oα</sub> (b). In addition, the mitral-tufted cell layer (MT) of the anterior and posterior AOB is stained with anti-G<sub>oα</sub>. Cx = frontal cortex. Bar = 200 μm.

receptors and effectors. G<sub>sα</sub> subunits stimulate and G<sub>12α</sub> subunits inhibit adenylate cyclase, respectively, and G<sub>oα</sub> subunits activate phospholipase C. Thus the differential distribution of these G proteins may relate directly to regional differences in function.

Eight adult (3 males, 5 females) Brazilian gray short-tailed opossums, *Monodelphis domestica*, were anesthetized with Metofane (Pitman-Moore, Mundelein, IL) inhalation and transcardially perfused with saline followed by Bouin's fixative, 4% paraformaldehyde and 15% picric acid or 4% paraformal-

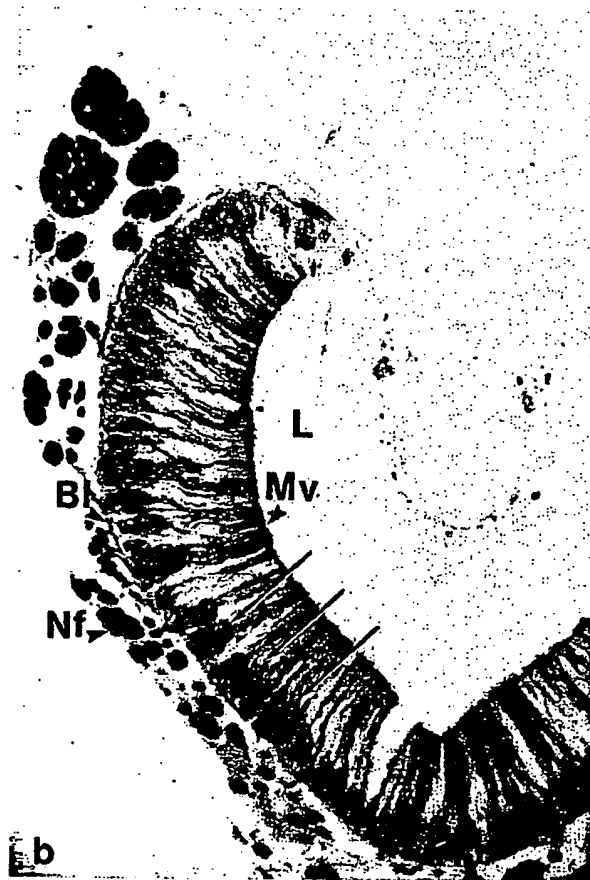
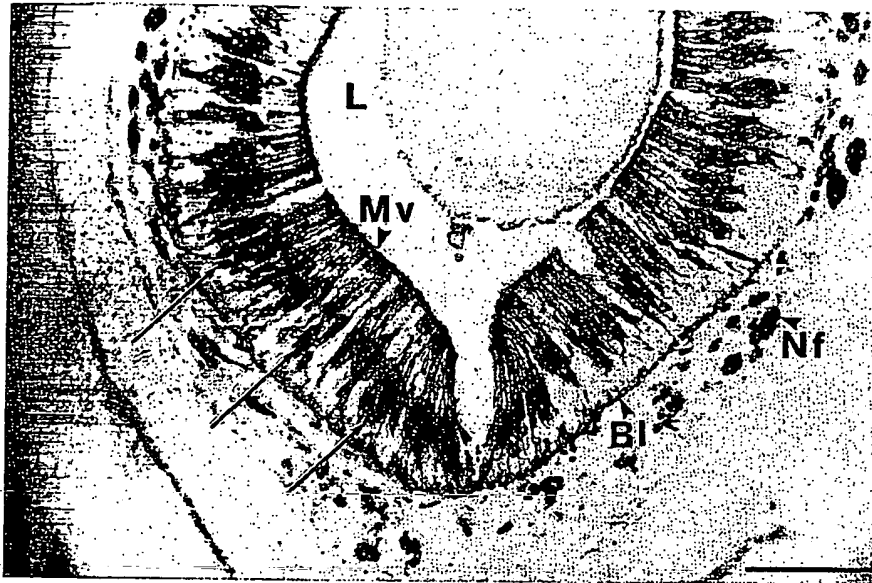
dehyde, 1% glutaraldehyde and 0.2% picric acid. The brains were post-fixed overnight, paraffin embedded, and 10 μm parasagittal sections cut and collected on subbed slides. A series was stained with Cresyl violet for morphological identification. The vomeronasal organs were dissected free of the surrounding bone, embedded in paraffin and cut in the parasagittal or coronal planes. Sections containing the accessory olfactory bulbs and vomeronasal organs were processed for G protein immunoreactivity.

Slides were deparaffinized, rehydrated and subjected to standard immunocytochemical ABC procedures with the appropriate Vectastain Elite kit (Vector Laboratories, Burlingame, CA) using 3,3'-diaminobenzidine and H<sub>2</sub>O<sub>2</sub> as the chromogen or with an additional intensification procedure (HistoMark Black, Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD). The primary rabbit antibodies to G<sub>12α</sub> and G<sub>oα</sub> were obtained from Dr. Tomiko Asano (Institute for Developmental Research, Kasugai, Aichi, Japan) and Upstate Biotechnology Inc., Lake Placid, NY. Antibodies were used at a concentration of 1:500 to 1:50,000. Specificity controls were performed by preabsorbing the primary antibodies with their appropriate antigens. Additional control sections were stained omitting the primary antibody.

Antibodies to G<sub>12α</sub> stained the nerve and glomerular layers of the anterior AOB only (Fig. 1a). Antibodies to G<sub>oα</sub> stained the nerve and glomerular layers of the posterior AOB, left the anterior AOB lightly stained depending on the concentration of antibody used, and stained a band corresponding to the mitral-tufted cell layer of both anterior and posterior parts of the AOB (Fig. 1b).

In the vomeronasal organ (VNO) both antibodies stained the nerve bundles running in the subepithelial mucosa. Antibodies to G<sub>12α</sub> stained the membranes of bipolar neurons whose cell bodies were located in the middle third of the epithelium. Their stained dendrites and axons were clearly visible and their luminal terminations were intensely stained (Fig. 2a). Antibodies to G<sub>oα</sub> stained a different population of bipolar neurons, those in the basal third of the epithelium (Fig. 2b). The axons and dendrites of these neurons were also well stained; however, the luminal terminations were more weakly stained than those stained with G<sub>12α</sub>. With both stains the bipolar neurons appeared to cluster in minicolumns. Good differential staining in the vomeronasal epithelium was achieved at a concentration of 1:5000 with each antibody.

Fig. 2. Coronal sections through the opossum vomeronasal organ immunostained with anti-G<sub>12α</sub> (a) and anti-G<sub>oα</sub> (b). Top is dorsal. The middle layer of the sensory epithelium is stained with anti-G<sub>12α</sub> (long arrows in a) and the basal third of the sensory epithelium is stained with anti-G<sub>oα</sub> (long arrows in b). The microvillar surface (Mv) of the epithelium and nerve fascicles (Nf) are stained with both antibodies. L = lumen; Bl = basal lamina. Bar = 100 μm.



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Sections treated with primary antibody preabsorbed with its respective antigen did not contain any differentially stained areas. Similarly, sections processed with the primary antibody omitted did not show any staining.

The results presented above for the glomerular and VN nerve layers of the AOB are similar to those reported in the rat [15]. In the opossum,  $G_o$  immunoreactivity was observed in the mitral-tufted cell layer of both the anterior and posterior AOB whereas in the rat the 'molecular' layer of the AOB, which we believe corresponds to Takami et al.'s [19] mitral-tufted cell layer, stains with both antibodies [15]. These apparently contradictory findings could represent true species differences.

Bipolar neurons in the middle third of the epithelium were  $G_i$ -immunoreactive (-ir), whereas those in the basal third were  $G_o$ -ir. There are no reports, to our knowledge, of a similar study in the vomeronasal epithelium of the rat, or any other mammal. In garter snakes  $G_i$  and  $G_o$  immunoreactivity is not spatially segregated in the vomeronasal epithelium [7]. In the rat olfactory epithelium only a sparse, broadly distributed population of bipolar neurons are  $G_i$ -ir, whereas a larger population of bipolar neurons are  $G_o$ -ir [16]. The locations of these immunoreactive neurons was not confined to any particular layer or area of the epithelium.

The laminar segregation of  $G_i$ -ir and  $G_o$ -ir bipolar neurons raises the issue of whether these cells differ in stage of maturation. Since the  $G_o$  population is more basally situated than the  $G_i$  population, it is possible that the former is less mature. In the olfactory epithelium of vertebrates [5] and the vomeronasal epithelia of snakes [21], the more apically situated bipolar neurons are more mature and better differentiated. In contrast, mouse [1] neurons are newly generated in the marginal zone of the VNO where the sensory and nonsensory epithelia meet and migrate from the margins to the center of the sensory epithelium as they mature. In regenerating mouse vomeronasal epithelium [2] repopulation of the sensory epithelium occurs from basal to apical levels, although the location of the stem cells in the regenerating epithelium has not been clearly identified. If the opossum VNO is constituted similarly to the regenerating mouse VNO, snake VNO or vertebrate olfactory epithelium, it is possible that the more basally situated  $G_o$ -ir neuronal population is 'younger' than the more apically situated  $G_i$ -ir neuronal population. However, it is unlikely that the more basally situated neurons are undifferentiated stem cells, the source of newly formed neuron, since the staining with  $G_o$  antibody reveals processes extending to the luminal surface, staining of the microvillar border and staining of the axon bundles in the subepithelial mucosa, all characteristics of mature bipolar neurons.

What is the relationship between the  $G_o$ -ir cells in the VNO and the  $G_o$ -ir posterior AOB? It is possible that the  $G_i$ -ir and  $G_o$ -ir neurons of the VNO project their axons to the anterior and posterior AOB, respectively. A retrograde tracing study is currently in progress to answer this question. Several examples of correspondence between biochemically identified cells in the main olfactory epithelium and their terminations in the MOB exist: Mab RB-8, in the rat [13] and Mab R4B12 in the rabbit [8] stain neurons in the ventrolateral portion of the olfactory epithelium and their axonal projections to the ventrolateral MOB. In contrast, in the rabbit AOB where two monoclonal antibodies differentially stain the rostralateral and caudomedial regions, retrograde tracing studies indicate that the bipolar neurons that project to the AOB are not topographically segregated in the epithelium [8]. Thus, there exist at least two patterns of distribution of biochemically identified receptor cells in the epithelium and their projections onto the bulb (see Schwob [12] for discussion): those in which there is a clustering of similar neurons in the epithelia and those in which neurons with similar biochemical characteristics and target zones are distributed throughout the epithelium.

The significance of heterogeneity in the chemoarchitecture of the accessory olfactory bulb has yet to be ascertained. Schwob [12] suggests that characterization of biochemically defined subsets of olfactory neurons may result in identification of neuronal subsets with similar sensitivity spectra to odorants. In rat and mouse, bipolar neurons expressing distinct receptors are segregated topologically in the olfactory epithelium [3,9,20]. However, within each topological zone several different types of receptors may be expressed and the neurons expressing these different receptors are randomly distributed within the circumscribed zone. Thus, in the olfactory epithelium a partial pattern of spatial segregation based on receptor type is present. It has yet to be demonstrated, however, that these receptor types are the basis of odor identification. In addition to differences in odor specificities, the differences observed in the distribution of G proteins, carbohydrate moieties and monoclonal antibodies may reflect functional heterogeneity related to transduction mechanisms and to cell-cell or cell-substrate adhesion patterns that are involved in axon-target interactions.

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